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New method for the determination of carbamate and pyrethroid insecticides in water samples using on-line SPE fused core column chromatography

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ABSTRACT

A new HPLC column-switching method using large volume sample injection and fused-core columns for on-line solid phase extraction have been developed for the determination of the following carbamates and pyrethroids: aldicarb, carbaryl, pirimicarb, carbofuran, kadethrin, flumethrin, fenpropathrin, fenoxycarb, tau-fluvalinate and fenvalerate, in surface water samples. Sudan I was used as internal standard. The proposed method was performed using 100 μl sample injection followed by an on-line solid phase extraction procedure and finally the compounds were identified and quantified by liquid chromatography with ultraviolet detection. The separation was carried out on C-18 reversed phase column based on fused-core particle technology. The influence of the injected sample volume, the variables affecting to SPE process and the conditions for the separation on an analytical column, were studied and optimized. The limits of detection ranged from 5.5 to 8.9 μg L^{-1}, and limits of quantification from 18.4 to 29.7 μg L^{-1}, while inter- and intra-day variability was under 15%. This new analytical procedure was satisfactorily applied for the determination of these organic pollutants in surface water samples located in Czech Republic. Concentration levels were found for some of these pollutants up to 26.11 μg L^{-1} in the river Elbe and up to 34.53 μg L^{-1} in the closed lakes samples.

1. Introduction

In 2007, the world pesticide amount used was estimated in 2.36 billion kg. Approximately 40% of this production corresponds to herbicides, 17% insecticides, 10% fungicides and 33% others [1]. Nowadays, there is an increasing public concern for environmental safety and for this reason it is necessary to study the presence of organic pollutants in different environmental compartments [2,3]. The use of large quantities of insecticides and pesticides in agriculture activities is one of the main causes of pollution of surface and ground water [4] and for this reason legislation is constantly changing and becoming more strict. The Water Framework Directive (WFD) (Directive, 2000/60/EC – European Parliament and Council of the European Union, 2000), have established the environmental quality standards (EQS) for pesticides, their relevant metabolites, degradation and reaction products in 0.1 μg L^{-1} for individual compounds and 0.5 μg L^{-1} for the sum of pesticides in ground water [5].

N-methylcarbamates are derived from carbamic acid and are extensively used in agriculture as insecticides, herbicides and fungicides [6]. Pyrethroids are synthetic insecticides based on the structure of the natural chemicals pyrethrins, which are produced by the flowers of pyrethrums and they are used as pesticides too. The use of carbamates is increasing due to they are less persistent in the environment than other pesticides such as pyrethroids, organophosphorous and organochlorine, however it is important to take into account that carbamates are highly biodegradable but more toxic than pyrethroids [7], and for example some carbamates such as carbofuran, are very toxic to the central nervous system and it is a strong endocrine disruptor affecting human and animal at low doses [8]. Ryan et al. determined the acute toxic effects of carbofuran on the levels of endocrine hormones in the serum of male Sprague-Dawley rats. When rats were exposed to an acute dose of carbofuran (1.5 mg kg^{-1}) showed the onset of cholinergic signs and when intensity was increased, toxic signs of maximal severity were observed within 30–60 min. The results suggest that an acute exposure to carbofuran may cause transient endocrine disruption [9]. Dana et al. evaluated in utero exposures to pesticides by measuring maternal and cord serum biomarkers in a New Jersey cohort of pregnant women and the birth outcomes of their neonates. Carbofuran was one of the pesticides most frequently detected in the serum samples. The results suggest that in utero exposures to certain pesticides may alter birth outcomes [10]. For this reason is

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necessary to develop new procedures where the sample treatment is simplified and an improved of the chromatographic techniques is carried out to detect these compounds in a low range of concentration.

In the present work the following compounds were analyzed: aldicarb, carbaryl, pirimicarb, carbofuran, kadethrin, flumethrin, fenpropathrin, fenoxycarb, tau-fluvalinate and fenvalerate. These compounds were chosen for our study due to they are used in the Czech republic and due to their toxicity is necessary to develop sensitive analytical methods for their determination in the environment.

In the literature many articles describe the determination of pesticides in environmental matrices. Some methods are based on the use of gas chromatography (GC) [11–13]. For example Yang et al. [14] proposed a sensitive and selective gas chromatographic mass spectrometric method, based on derivatization with 9-xanthydrol for the simultaneous determination of five carbamate pesticides in surface water, and the limits of quantification (LOQ) were in the range of 0.007–0.028 $\mu g\,L^{-1}$, but in this study none of analytes were detected in samples. However the majority of methods published are based on the use of liquid chromatography (LC). Different detection techniques were used such as: (1) ultraviolet detection [15], for example Hogendoorn et al. proposed both a screening method for the determination of acidic pesticides in four types of soils, based on the use of microwave assisted solvent extraction and coupled-column reversed-phase liquid chromatography (LC–LC) with UV detection at 228 nm. Recoveries between 60 and 90% were obtained and LODs were between 5 and 50 $mg\,kg^{-1}$ [16] and an analytical method for the determination of polar pesticides in water using coupled column RPLC with UV detection and injecting 4 mL of sample was proposed. The limits of determination for pesticides such as bentazone and isoproturon were 0.1 $pg\,L^{-1}$ [17]; (2) fluorescence detection [18], for example García de Llasera et al. studied the presence of pesticides in ground and surface waters from an agricultural zone in northwest Mexico. Trace determinations were made by liquid chromatography with post-column fluorescence detection. Level of contamination with methiocarb was 5.4 $\mu g\,L^{-1}$ in a ground water sample and for 3-hydroxycarbofuran was 18 $\mu g\,L^{-1}$ in a surface water sample [19]. Vassilakis et al. [20] evaluated the extraction of triazines, organochlorine, carbamates and acidic pesticides from surface and ground water from Greece, using gas chromatography with selected detection methods (electron-capture detection, nitrogen–phosphorus detection, mass spectrometry) and liquid chromatography-post-column derivatization fluorescence detection. Recoveries varied from 52 to 102%. The limit of detection for seventeen organochlorine compounds was better than 0.003 $\mu g\,L^{-1}$ and the limit of detection for other 15 analytes was better than 0.06 $\mu g\,L^{-1}$; (3) and mass spectrometry detection [20–23], such as Kampioti et al. [24] developed an automated method for the determination of twenty pesticides in natural and treated waters using on-line solid phase extraction and liquid chromatography mass spectrometry. Limits of detection between 0.004 and 2.8 $ng\,L^{-1}$ were obtained. García-Ac et al. [25] developed an on-line solid phase extraction of large-volume injections coupled to liquid chromatography tandem mass spectrometry method for the simultaneous quantitation and confirmation of 14 selected trace organic contaminants in drinking and surface water. Extraction recoveries ranged from 60 to 109% and the method detection limits ranged from 0.6 to 6 $ng\,L^{-1}$. Although several methods have been published for determination of these compounds in the environment, due to the complexity of matrices and they are present in trace levels, sample pre-treatment like as soxhlet extraction, ultrasonic assisted extraction, microwave extraction, pressurized liquid extraction, super-critical fluid extraction, etc., is required, but these extraction techniques present several disadvantages due to they are tedious, time consuming and consuming large amounts of organic solvent. On the other hand, sensitive detection is required and although the use of MS detectors allows to obtain good sensitivity, however it is expensive [26]. These reasons along with trends are focused on the application of green chemistry principles [27] have allowed the development of improved chromatographic techniques, e.g., column switching chromatography, on-line SPE and liquid chromatography and multidimensional chromatography [28–30]. A remarkable decrease in total analysis time can be achieved with the introduction of the on-line SPE step in which the loading of the sample into the cartridge, the clean-up of the matrix, the extraction of target analytes and their determination is automatically coupled in one chromatography system.

Today, the new approach in fast chromatography and high efficiency separations in conventional HPLC systems is using columns with fused-core particles technology. These columns are packed with porous shell silica particles consisting of 1.7 μm fused-core, 0.5 μm layer of porous silica coating and 2.7 μm total particle diameter. This column technology enables the shortening of the diffusion path for analytes, which allows rapid mass transfer and, thus, reduced axial dispersion and peak broadening. Fused-core column technology offers the following advantages: improvement of the efficiency of the separation process over fully porous particles, its efficiency of separation is comparable to totally porous sub-2 μm particle size columns but with lower back-pressures and it allows to work with higher flow rates [31].

Notably, there is important to highlight that only a few papers concerning the on-line SPE-HPLC coupled with fused-core columns are described in the literature. For example, an analytical method based on an on-line solid phase extraction coupled to liquid chromatography with fluorescence detection has been developed to determine quinolones in tap water and human urine by Lara et al. [32]. A C-18 column containing core–shell particles (2.6 μm) was used to achieve peak efficiencies up to 200,000 plates/m and at a flow rate of 1.2 mL min^{-1}. The limits of detection were ranging between 7 and 110 $ng\,L^{-1}$. Gallart-Ayala et al. [33] proposed an on-line column-switching LC–MS/MS method to analyze bisphenol A and its chlorinated derivatives in water. Fast liquid chromatographic separation was performed on a C-18 reversed phase column based on fused-core particle technology (2.7 μm particle size) providing analysis times shorter than 3 min and high peak efficiencies, and Wode et al. [34] described a multiresidue method for the simultaneous quantification of 72 micropollutants in aqueous samples by ultra high performance liquid chromatography–high resolution mass spectrometry (UHPLC–HRMS). A sample volume of 1 mL was enriched by on-line SPE, separated on a 2.6 μm core–shell column and detected with high resolution mass spectrometer. Limits of quantification ranged between 0.01 and 0.06 $\mu g\,L^{-1}$ in drinking water, 0.03 and 0.38 $\mu g\,L^{-1}$ in diluted surface water, 0.06 and 0.38 $\mu g\,L^{-1}$ in diluted wastewater treatment plant effluent.

From this point of view, the idea of fused-core columns for on-line solid phase extraction of samples is highly innovative and not well described. Therefore, on-line coupling of fused-core sorbents to conventional HPLC instruments shows a novel and promising approach for future instrumental applications in column-switching systems.

The aim of this work is, for the first time presented and new method for the determination of carbamates and pyrethroids in surface water samples employing large volume sample injection and column-switching technique using an on-line sample preparation and separation in one step. Compounds with a wide range of physicochemical properties, pyrethroids and carbamates with very different log P values, were simultaneously extracted and separated. The influence of the injected sample volume, the variables affecting SPE process and the conditions for the

separation in an analytical column, were studied and optimized. After validation, the method was successfully applied in the analysis of surface water samples obtained from the river Elbe and the closed lakes.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were of analytical grade unless otherwise specified. Individual standard of carbamates and pyrethroids: aldicarb (purity 99.9%), carbaryl (purity 99.98%), carbofuran (purity 99.9%), fenoxycarb (purity 99.6%), fenpropathrin (purity 99.1%), flumethrin (purity 95.9%), fenvalerate (purity 98.6%), kadethrin (purity 90.8%), pirimicarb (purity 98.5%), T-fluvalinate (purity 91.6%) and Sudan I (purity 97.0%) used as internal standard, were supplied by Sigma-Aldrich Chemie (GmbH, Germany). The ultra-pure water was purified through a Milli-Q (Millipore, Bedford, MA, USA). Polytetrafluoroethylene (PTFE) filters (pore size 0.45 μm, and 25 mm in diameter) were supplied by Trading New Technologies S.A (USA).

2.2. Instrumentation and software

Analysis were performed using a Shimadzu Prominence system (Shimadzu Corporation, Kyoto, Japan), high-performance liquid chromatography system equipped with solvent delivery systems LC-20AD, with a SIL-20AC Autosampler, DGU-AS online degasser, SPD-M20A DAD detector, CTO 20AC column oven with FCV-12AH high pressure six-port switching valve and CBM-20A communication module. The system control, data acquisition and data evaluation were performed by Shimadzu "LC Lab-Solution" software (Shimadzu Corporation, Kyoto, Japan). Stat graphics 5.0 software package was used for statistical and regression analysis [35].

2.3. Sample collection

River water samples were collected from the river Elbe located in Hradec Králové (East of the Czech Republic). A total of 1.5 L samples was collected and kept in amber glass bottles. Lake water samples were collected from the lake Třebeš, from the southeast part of the city center of Hradec Králové. A total of 1.5 L samples was collected and kept in amber glass bottles. All of them were stored refrigerated in the dark at 4 °C until analysis.

2.4. Preparation of stock solutions and samples

Individual stock standard solutions of carbamates and pyrethroids were prepared by dissolving of substance in acetonitrile in concentration 500 mg L^{-1}. Standard stock solutions were stored at 4 °C in the dark, remaining stable for at least six months. Working standard solutions were prepared immediately before use by appropriate dilution in 5% acetonitrile in water. The calibration standard solutions were prepared in the concentration range of 25–250 μg L^{-1}, using five calibration points. All solutions were kept in amber glass bottle.

Prior to injection into the LC system, five milliliters of surface water samples were filtered through PTFE filters. A 5% (v/v) of acetonitrile was added to filtered aqueous samples and 100 microliters of sample were injected directly into the HPLC system.

2.5. HPLC column switching analysis

A column-switching HPLC system was used for the simultaneous preconcentration and determination of the analytes. The sample preconcentration was carried out using a precolumn Ascentis Express-C-18 (0.5 cm × 4.6 mm I.D., 5 μm particle size) from Supelco, (Sigma-Aldrich, Germany), with a washing mobile phase (10% methanol in water) at a flow rate of 1.0 mL min^{-1}.

Chromatographic separation of both carbamates and pyrethroids was performed using an Ascentis Express C-18 (fused-core) analytical column (10 cm × 4.6 mm I.D., 5 μm particle size) from Supelco (Sigma-Aldrich, Germany) using a gradient mobile phase consisting of water (solvent A) and acetonitrile (solvent B). Firstly, in the extraction dimension an on-line preconcentration was carried out and 100 μL of sample with 5% acetonitrile was directly injected onto precolumn Ascentis Express-C-18, where interferences and sample matrix were removed to waste. Washing mobile phase containing 10% methanol in water was pumped for 1.0 min at a flow rate of 1.0 mL min^{-1}. The analytical column was equilibrated using the analytical mobile phase at this time. Valve switch for the analytes transfer from the extraction column to analytical column was set at 1.0 min and it was found as the optimal time for sufficient sample clean-up. Separation and analysis of the analytes were performed with an interval from 3.0 to 22.0 min. Zone of preconcentrated sample was transported in back-flush direction from preconcentration column onto analytical column Ascentis Express C-18, where the analysis was performed with the following gradient: $t=2$ min, 65% A; $t=$ 12 min, 35% A; $t=17$ min, 20% A. Then amount of A was set at 65% (v/v) for 11 min to restore the initial conditions and for column conditioning. The column oven temperature was kept constant at 30 °C for both columns. The detector wavelength was set at 210 nm. Total run time was 28 min.

3. Results and discussion

3.1. Optimization of on-line SPE conditions

To optimize the on-line SPE step, there was necessary to select: the preconcentration column (type and chemistry of the sorbent), composition of the washing mobile phase (water-organic solvent ratio), flow rate, washing time and volume of sample injection, selecting the optimal conditions to obtain a strong retention of analytes on column and matrix component interference elution.

The main goal was to obtain an improvement in sensitivity, selectivity and peak shapes using shorter chromatographic times. The following preconcentration columns were tested: short Guard Cartridge Ascentis Express C-18 (0.5 cm × 4.6 mm I.D., 5 μm particle size) from Supelco, (Sigma-Aldrich, Germany); Monolithic column C-18 (5 cm × 4.6 mm I.D.) and Monolithic column C-18 (5 cm × 3 mm I.D.) both from Phenomenex (USA). Guard Cartridge Ascentis Express-C-18 was selected for our study. Although monolithic columns provided in general a good extraction for analytes investigated, after some injections we observed the problems with back-pressure inconsistency between monolithic extraction column and fused-core analytical column. Moreover the dispersion of the zone of analytes were higher comparing with short fused-core precolumn during the extraction step. The effect of different compositions of washing mobile phase with acetonitrile ranging from 5% to 30% (v/v) with water and methanol ranging from 5% to 30% (v/v) with water was analyzed. The tested range of acetonitrile solutions in washing mobile phase showed weak retention of polar carbamates on fused-core extraction sorbent. Increasing acetonitrile concentration resulted in decreasing peak areas of early eluted carbamates. Moreover, the peak shape of polar carbamates tended to tail on second separation column due to dispersion on extraction column. Therefore methanol was chosen as organic solvent in mixture with water because all analytes were sufficiently retained on the guard cartridge column and eluted from the precolumn with using analytical mobile phase under the

gradient conditions without tailing and decreasing peak areas. A compromise in methanol content in washing mobile phase was necessary to find. When the percentage of methanol increased in mobile phase, the absorbance signal intensity increased for carbamates but the intensity signal decreased for pyrethroids. For these reasons, the washing mobile phase containing 10% (v/v) of methanol in water was selected for further experiments. Later, the influence of flow rate in a range from 0.4 to 1 mL min^{-1} and washing times for extraction column ranging from 0.5 to 2 min were tested. A flow rate of 1.0 mL min^{-1} and column-switch time of 1 min were selected as optimal values to remove the interferences from the matrix and to obtain sufficient retention of all analytes. The last step, a study to improve the sensitivity and peak shape by testing the different injection of sample volumes was performed. A range from 100 to 1500 μL was analyzed, and 100 μL was chosen as the injection volume providing the optimal Gaussian peaks. Moreover, a linear relationship between peak area of carbamates and injection volume of sample was decreasing when the sample volume was bigger than 100 μL. The breakthrough volume for the mostly polar carbamate aldicarb was set on 100 μL of sample in 5% acetonitrile solution. Therefore 100 μL of sample injection in 5% acetonitrile solution and 1 min guard column washing with 10% methanol in water at flow rate of 1.0 mL min^{-1} were found to be optimal for sample preparation and preconcentration prior to carbamates and pyrethroids separation in the analytical column.

3.2. Optimization of separation conditions on analytical column

The following analytical columns with different chemistry and structure of stationary phases – core–shell particle column were tested: Ascentis Express C-18 (10 cm × 4.6 mm I.D., 2.7 μm particle size); Ascentis Express C-18 (10 cm × 4.6 mm I.D., 5 μm particle size); Ascentis Express RP-Amide (10 cm × 4.6 mm I.D., 2.7 μm particle size) and Ascentis Express Phenyl-hexyl (10 cm × 4.6 mm I.D., 2.7 μm particle size) from Supelco (Sigma-Aldrich, Germany). The last tested column was a multi-layered silica-organic hybrid stationary phase – YMC-Triart C-18 (10 cm × 4.6 mm I.D., 5 μm particle size) from YMC Europe Gmbh.

Firstly, the column Ascentis Express RP-Amide was tested. Its principle retention mode is based on reversed phase with embedded polarity and the principle solute interaction is through hydrophobic and H-bonding. RP-Amide phase reduces silanol interactions with basic analytes and it provides increased selectivity for polar compounds, especially those that can act as a hydrogen bond donor. Although a good selectivity and separation was obtained for all carbamates not all pyrethroids showed a good retention and separation.

Second, the column Ascentis Express Phenyl–Hexyl was tested. Its principle retention mode is based on reversed phase with pendant aromaticity and the principle solute interaction is through hydrophobic and π–π mechanisms. Phenyl–Hexyl stationary phase shows an unique reversed-phase selectivity, especially for polar aromatics and heterocyclic compounds, arising from solute interaction with the aromatic ring and its delocalized electrons. This column showed under the tested conditions poor resolution for both group of compounds – carbamates and pyrethroids.

Third, a fully porous column YMC-Triart C-18 was tested. This column has a multi-layered silica/organic hybrid stationary phase and due to its unique particle composition, a balanced hydrophobicity and silanol activity is achieved. This column showed in general a bad retention and peak broadening for carbamates and pyrethroids analyzed. The advantage of this column was very low flow resistance in HPLC system only. The retention of compounds on the column was lower compared to C-18 fused-core stationary phases.

Finally, columns Ascentis Express C-18 with different particle sizes (2.7 vs. 5 μm) were tested. Its principle retention mode is based on reversed phase and the principle solute interaction is hydrophobic (dispersive) interactions. The best results were obtained with the column Ascentis Express C-18 (10 cm × 4.6 mm I.D., 2.7 μm particle size), but problems with back-pressure appeared during the large volume sample injection method optimization. Then was decided to test the column Ascentis Express C-18 (10 cm × 4.6 mm I.D., 5 μm particle size). In this case a good separation and resolution of all compounds was obtained and furthermore the problems with back-pressure were removed. Therefore the column Ascentis Express C-18 (10 cm × 4.6 mm I.D., 5 μm particle size) was chosen because better separation and resolution was obtained for all compounds analyzed. For all columns, different isocratic elution were tested with acetonitrile/water mobile phases ranging from 50% to 90% (v/v) of acetonitrile in water, but in isocratic mode only a few analytes were separated. Therefore, different gradient elution was tested. Analytical separation of carbamates and pyrethroids was carried out using a gradient mobile phase consisting of water (solvent A) and acetonitrile (solvent B), the optimized conditions were discussed in the Section 2.5. A compromise solution was necessary to get because the optimal conditions to separate all carbamates were not adequate to separate all pyrethroids and vice versa. Later, the influence of the flow rate in a range from 0.5 to 1.5 mL min^{-1} and the column temperature in a range from 30–60 °C for both columns (precolumn and analytical column) were tested. When flow rates were less than 1 mL min^{-1} analysis time increased but resolution of carbamates was not improved, and when the flow rates were higher than 1 mL min^{-1} the peaks of some compounds were overlapping. The column temperature was studied in a range from 30 to 60 °C for both columns. Resolution did not improve for any compound with higher temperatures, only pressure of the system was lower. A better separation of all analytes was obtained with a flow rate of 1 mL min^{-1}, and 30 °C for both columns. Fig. 1 shows the chromatogram obtained for a spiked water sample with a

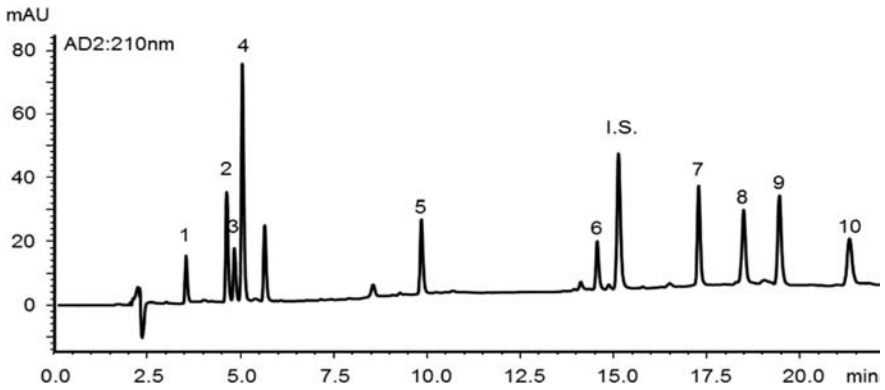

Fig. 1. Chromatogram obtained for a spiked water sample with a mixture of standards of carbamates and pyrethroids (500 μg L^{-1}). (1) aldicarb; (2) carbofuran; (3)pirimicarb; (4) carbaryl; (5) fenoxycarb; (6) Kadethrin; (I.S) Internal standard; (7) fenpropathrin; (8) fenvalerate; (9) tau-fluvalinate; (10) flumethrin.

Table 1
HPLC column-switching system suitability parameters.

Compounds	Retention time (min)	Retention time repeatability RSD (%)[a]	Repeatability of peak areas RSD (%)[a]	Retention factor (k')	Peak symmetry	Resolution
Aldicarb	3.42	0.18	0.98	0	1.41	0
Carbofuran	4.52	0.11	0.60	0.32	1.34	7.98
Pirimicarb	4.72	0.11	0.67	0.38	1.29	1.40
Carbaryl	4.94	0.11	2.64	0.44	1.36	1.54
Fenoxycarb	9.75	0.05	0.54	1.84	1.31	32.0
Kadethrin	14.47	0.03	0.77	3.22	1.32	29.01
Fenpropathrin	17.18	0.03	2.78	4.01	1.31	11.65
Fenvalerate	18.39	0.03	4.5	4.36	1.16	6.42
T-fluvalinate	19.35	0.03	0.96	4.64	1.24	4.71
Flumethrin	21.24	0.02	0.85	5.19	1.15	8.36

[a] R.S.D. for repeated injections of standard solution ($n=5$).

Table 2
Quality and statistical parameters.

	b (L μg^{-1})	s_b (L μg^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	LDR[a] (μg L^{-1})	r^2
Aldicarb	2×10^{-4}	2×10^{-6}	7.4	24.6	24.6–250.0	0.99917
Carbofuran	4×10^{-4}	3×10^{-6}	5.5	18.4	18.4–250.0	0.99954
Pirimicarb	2×10^{-4}	2×10^{-6}	7.1	23.6	23.6–250.0	0.99924
Carbaryl	1×10^{-4}	1×10^{-6}	8.0	26.8	26.8–250.0	0.99902
Fenoxycarb	4×10^{-4}	5×10^{-6}	8.4	28.2	28.2–250.0	0.99892
Kadethrin	2×10^{-4}	3×10^{-6}	7.1	23.8	23.8–250.0	0.99923
Fenpropathrin	5×10^{-4}	7×10^{-6}	8.9	29.7	29.7–250.0	0.99880
Fenvalerate	5×10^{-4}	7×10^{-6}	8.8	29.3	29.3–250.0	0.99883
Tau-fluvalinate	5×10^{-4}	6×10^{-6}	7.7	25.8	25.8–250.0	0.99909
Flumethrin	3×10^{-4}	3×10^{-6}	6.2	20.8	20.8–250.0	0.99941

[a] Each concentration was measured in triplicate; b – slope of the calibration curve; s_b – slope deviation; r^2 – correlation coefficient; LOD – limit of detection; LOQ – limit of quantification; LDR – linear dynamic range.

mixture of standards of carbamates and pyrethroids using column switching technique.

3.3. Analytical performance and validation of the method

Calibration graphs for samples treated according to Section 2.4 were built by injecting different standard solutions in the range of 25.0–250 μg L^{-1}. First, the method was applied to blank surface water samples to confirm the absence of target compounds within the LOD of the method. Calibration curves were built using the analyte/internal standard peak area ratio versus analyte concentration. Sudan I was used as internal standard.

In the validation of any analytical method, two fundamental aspects need to be examined: the limits of detection (LOD) and the limits of quantification (LOQ). In this paper, these parameters were calculated by taking into consideration the standard deviation of residual $S_{y/x}$, the slope b of the calibration curve and an estimate S_0 obtained by extrapolation of the standard deviation of the blank [36]. The LOD was $3S_0$ and the LOQ was $10S_0$. The LOD ranged from 5.5 to 8.4 μg L^{-1} for carbamates, and ranged from 6.2 to 8.9 μg L^{-1} for pyrethroids. The LOQ ranged from 18.4 to 28.2 μg L^{-1} for carbamates and ranged from 20.8 to 29.7 μg L^{-1} for pyrethroids. Linearity of the calibration graphs was tested according to the Analytical Methods Committee guidelines [37]; The P values of the *lack-of-fit* test, P_{lof} (%), were greater than 5% in all cases with three replicates and three injections of each standard, indicating that the data are well modeled by a line in all cases. The behavior of carbamates and pyrethroids was linear in a range LOQ – 250 μg L^{-1}, with r^2 values close to 1.0 for each compound analyzed. Table 1 summarizes chromatography system suitability parameters and in Table 2 quality and statistical parameters are shown.

Table 3
Accuracy of the method. Precision and trueness of target compounds in samples.

	Spiked[1] (μg L^{-1})	Found ± SD (%, RSD)[a] (μg L^{-1})	Recovery (%)
Aldicarb	50	52 ± 2 (4)	104
	100	100 ± 4 (4)	100
	250	253 ± 6 (2)	101
Carbofuran	50	51 ± 3 (5)	102
	100	100 ± 3 (3)	100
	250	250 ± 7 (3)	100
Pirimicarb	50	49 ± 2 (4)	98
	100	98 ± 4 (4)	98
	250	248 ± 4 (2)	99
Carbaryl	50	50 ± 3 (5)	99
	100	100 ± 3 (3)	100
	250	255 ± 4 (2)	102
Fenoxycarb	50	50 ± 2 (3)	100
	100	99 ± 5 (5)	99
	250	258 ± 6 (2)	103
Kadethrin	50	50 ± 3 (5)	101
	100	99 ± 4 (4)	99
	250	261 ± 9 (3)	105
Fenpropathrin	50	50 ± 2 (3)	99
	100	100 ± 1 (1)	100
	250	253 ± 7 (3)	101
Fenvalerate	50	50 ± 2 (5)	100
	100	103 ± 2 (2)	103
	250	250 ± 10 (4)	100
Tau-fluvalinate	50	50 ± 2 (4)	100
	100	101 ± 5 (5)	100
	250	259 ± 10 (4)	104
Flumethrin	50	50 ± 2 (4)	100
	100	100 ± 5 (5)	100
	250	255 ± 10 (4)	102

[a] Mean of 7 determinations; SD: standard deviation; RSD: relative standard deviation.

On the other hand, the accuracy of the method in terms of trueness and precision was also studied. Due to the absence of certified materials, a recovery assay was performed in order to validate the method in terms of trueness. Blank surface water samples were analyzed to ensure that they did not contain the analytes or they were below the LOD of the method. Trueness was evaluated by determining the recovery of known amounts of the tested compounds in surface water at three concentration levels (50.0, 100.0 and 250.0 μg L^{-1}).

Samples were analyzed using the proposed method and the concentration of each compound was determined by interpolation from the standard calibration curve within the linear dynamic range and compared with the amount of analytes previously added to the samples. The obtained recoveries are shown in Table 3. The recoveries were very close to 100% (ranged from 97.5% to 103.1% for carbamates and from 99.1% to 104.5% for pyrethroids). To evaluate the overall precision of the method, intra- and inter-day precision (as relative standard deviation, RSD) were assessed at the three concentration levels. The procedure was repeated three times on the same day to evaluate repeatability and was repeated for seven consecutive days to determine inter-day reproducibility. Repeatability and inter-day reproducibility values (RSD) are summarized in Table 3. The accuracy of the proposed methodology was demonstrated with precision and recovery values.

Table 4
Results obtained for carbamates and pyrethroids in the analysis of surface water samples.

Concentrations ($\mu g\ L^{-1}$)		
Analytes	**Lake Třebeš**	**River Elbe**
Aldicarb	< LOD	< LOD
Carbofuran	< LOD	< LOD
Pirimicarb	D	D
Carbaryl	< LOD	< LOD
Fenoxycarb	D	D
Kadethrin	D	26.11
Fenpropathrin	D	< LOD
Fenvalerate	34.53	< LOD
Tau-fluvalinate	D	< LOD
Flumethrin	D	D

[1]Mean of 6 determinations; LOD: limit of detection; D: compound detected.

3.4. Application to surface water samples

The new proposed method was applied to the determination of carbamate and pyrethroid insecticides in surface water samples collected from the river Elbe and lake Třebeš in city part Hradec Králové (Czech Republic). Table 4 shows the concentration values found for carbamates and pyrethroids in surface water samples from the lake Třebeš and the river Elbe. The concentration values were calculated for six replicate samples and concentration of each analyte was determined by interpolation in its standard calibration curve within its linear dynamic range. When water samples from the lake Třebeš were analyzed, concentration of three carbamates (aldicarb, carbofuran and carbaryl) were below the detection limit, two carbamates (pirimicarb and fenoxycarb) were detected but not quantified and in the case of pyrethroids four of them (kadethrin, fenpropathrin, tau-fluvalinate and flumethrin) were detected and only fenvalerate was quantified and its found concentration was 34.53 $\mu g\ L^{-1}$. Water samples from the river Elbe were analyzed, concentration of three carbamates (aldicarb, carbofuran and carbaryl) were below the detection limit, two carbamates (pirimicarb and fenoxycarb) were detected but not quantified, in the case of pyrethroids, three of them (fenpropathrin, fenvalerate and tau-fluvalinate) were below the detection limit, flumethrin was detected and only kadethrin was found with a concentration of 26.11 $\mu g\ L^{-1}$. When comparing the results obtained for water samples collected in the lake and in the river, in both cases the same compounds are found below the detection limit (aldicarb, carbofuran and carbaryl) and the same carbamates were only detected (pirimicarb and fenoxycarb), nevertheless in the case of pyrethroids only flumethrin was detected in both

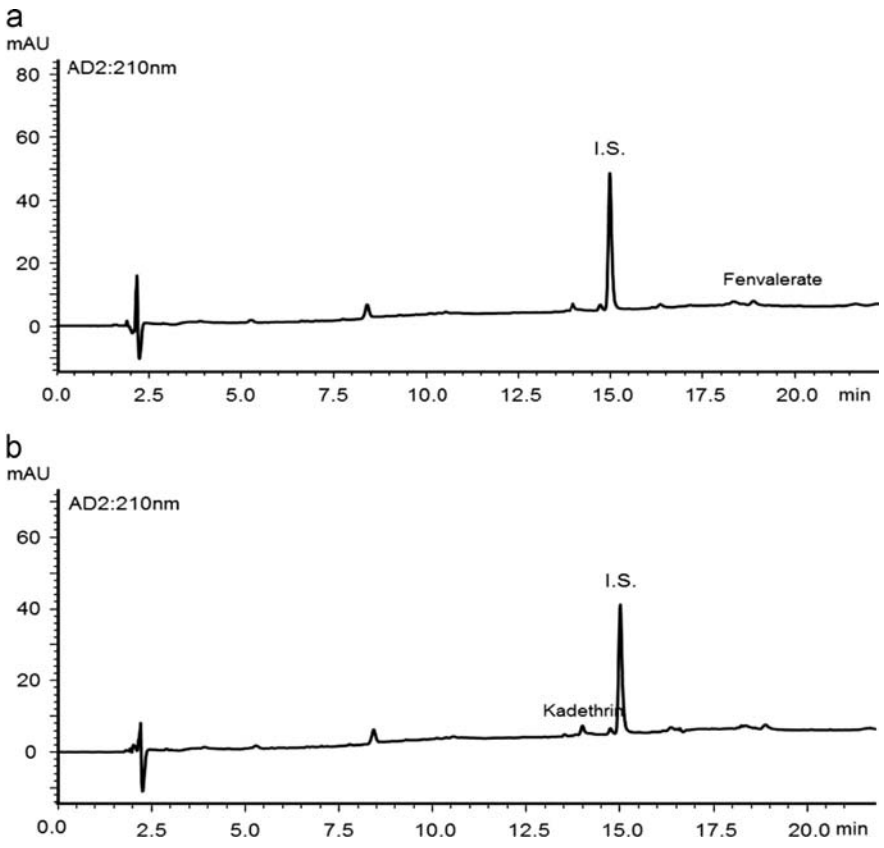

Fig. 2. Chromatogram of a lake water sample (a) and a river water sample (b).

classes of water samples and its concentration was found higher in lake samples than in river samples.

A representative chromatogram of a lake water and a river water samples are depicted in Fig. 2.

4. Conclusions

In the present work, a new method based on large volume sample injection with on-line SPE coupled to liquid chromatography and ultraviolet detection has been proposed. The main advantages of this method proposed are: (1) injection of relatively large volume sample allows to determine analytes at low concentrations using a conventional detector like as ultraviolet detection, (2) on-line sample pretreatment allows reduction of time and sample manipulation during sample treatment and (3) fast analysis using fused-core column technology in both dimensions of the chromatography system. Due to the column switching system, method allowed an automated and fast sample preparation step, and showed advantages compared to a conventional off-line SPE method performed manually. The risk of analytes loss or contamination were decreased as well, since the separation and sample extraction took place in one closed and automated system. The fused-core SPE column was used repeatedly under the proposed experimental conditions for over 500 injections of 100 μl of standard or environmental water samples.

Analytical performance of this method was validated and the method was successfully used for determination of carbamates and pyrethroids in surface water samples collected from city part Hradec Králové (Czech Republic). The results obtained for both classes of surface water samples show that in the case of carbamates, only pirimicarb and fenoxycarb were detected and in the case of pyrethroids, when lake water samples were analyzed, kadethrin, fenpropathrin, tau-fluvalinate and flumethrin were detected and only fenvalerate was quantified (34.53 $\mu g\ L^{-1}$), and in river water samples analyzed, flumethrin was detected and only kadethrin was quantified (26.11 $\mu g\ L^{-1}$).

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